Genetic Control of Incubation Behavior in the Domestic Hen

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ABSTRACT The genetic control of incubation behavior was investigated in the domestic hen by analysis of the incidence of the behavior in reciprocal crosses between nonbroody White Leghorn (WL) and broody Bantam (B) lines and in a backcross of F_1 males (WL male × B female) and WL females. The hypothesis tested was that a sex-linked gene (or genes) plays a dominant role in the expression of incubation behavior. The incidence of incubation behavior was tested in hens held in floor pens with access to nests containing hard-boiled eggs during a 28-wk photoinduced laying cycle. The cycle was repeated if the behavior was not observed during the first cycle. The incidence of incubation behavior in B and WL hens was 78.6% (n = 28) and 0% (n = 28), respectively. Contrary to prediction, the incidence of incubation behavior in the WL male × B female and the B male × WL female crosses were not significantly different (61.6%, n = 73; and 56.8%, n = 37, respectively). The incidence of incubation behavior in the F_1 backcross was 5.8% (n = 103), which was significantly less ($P < 0.001$) than predicted (39.3%). It was concluded that incubation behavior was not controlled by major genes on the Z chromosome. It was hypothesized that at least two dominant autosomal genes are involved, one causing and the other inhibiting the behavior with equal influence.

(*Key words*: broodiness, domestic chicken, female reproductive performance, incubation behavior, genetics)

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INTRODUCTION

Incubation behavior in domestic chickens consists of persistent nesting, turning and retrieval of eggs, characteristic clucking, and defense of the nest. If chicks hatch, the hen will brood and defend them and continue the characteristic clucking of incubating hens. Collectively, incubation and brooding behaviors are described as broodiness, although the term "broody" is often used to describe incubation behavior only. This usage reflects the fact that in commercial conditions, incubating hens are more likely to be encountered than those showing brooding behavior. Incubation behavior is associated with the cessation of egg laying, and selection for persistency of egg production has resulted in a reduction in the incidence of the behavior (Hutt, 1949). This reduction is particularly marked in Mediterranean breeds such as the White Leghorn (WL) in which incubation behavior is rarely observed. Selection experiments have demonstrated that the incidence of incubation behavior can be readily reduced, but it is difficult to eradicate completely (Hays and Sanborn, 1939).

All authors agree that incubation behavior is a polygenic trait but although some authors have presented evidence of contributory sex-linked genes (e.g., Saeki, 1957; Saeki and Inoue, 1979), others have concluded that incubation behavior is controlled by a small number of dominant autosomal genes with no sex-linkage (e.g., Goodale et al., 1920; Hays, 1940). Evidence for sex-linkage rests on the observation that the incidence of broodiness in reciprocal crosses between broody and nonbroody (WL) genotypes is lowest when the sire is a WL (Saeki, 1957; Saeki and Inoue, 1979). Some of the best evidence against the involvement of major sex-linked genes in incubation behavior comes from an analysis of the incidence broodiness within families showing differences in expression of the trait (Hays, 1940). In these studies, sires and dams were found to transmit the trait equally to their female offspring in a manner characteristic of their family.

Observations on the incidence of incubation behavior in reciprocal crosses may be complicated by the phenomenon of deferred broodiness. In this condition, broodiness may not be observed in the first laying year but is observed in the second or third laying cycles (Hays, 1940). It has been suggested that this condition may be particularly prevalent amongst the offspring of WL males mated to broody genotype dams (Hays, 1940). This suggestion may

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Abbreviation Key: B = Bantam; WL = White Leghorn.

explain the observations of Saeki (1957) and Saeki and Inoue (1979) on the differences in the incidences of broodiness in their reciprocal crosses as their hens did not appear to have been tested for the occurrence of broodiness for more than one laying cycle.

Interest in the possibility of sex-linked inheritance of broodiness has been prompted by the cloning of the chicken prolactin receptor gene (Tanaka et al., 1992) and its mapping to the Z chromosome (Dunn et al., 1998). The expression of incubation behavior in the chicken is associated with increased plasma prolactin (Sharp et al., 1988), which is thought to induce the behavior through prolactin receptor in the brain (Buntin, 1996). Support for this conclusion in the chicken comes from the demonstration of prolactin receptor mRNA in the basal and anterior hypothalamus of the domestic hen (Ohkubo et al., 1998).

The purpose of this study was to reinvestigate the possibility that the expression of incubation behavior may be partly controlled by major sex-linked genes. Particular attention was given to the possibility that hens may not express the behavior until they have experienced a second period of egg production. Observations were made on the incidence of incubation behavior in reciprocal crosses between Bantams (B) showing a high incidence of the behavior, and WL from a line, which has never been observed to show the behavior. The incidence of incubation behavior was also recorded in a F_1 backcross, male F_1 (male WL × female B) mated to a WL dam, to test the prediction that in this backcross, a dominant sex-linked gene for incubation behavior would result in a 50% incidence of the behavior. Romanov et al. (1999) reported a preliminary account of this work.

MATERIALS AND METHODS

Experimental Chickens and Matings

The experimental hens were from the B and WL flocks of the Roslin Institute. The B flock had been maintained for 18 yr for physiological studies on broodiness as a heterologous outbred population. The birds were of mixed ancestry, which was reflected in various feather and skin colors and a variable incidence of five toes (polydactyly). The WL flock (Line M71) had been maintained for 10 yr as a homologous inbred population for research on chicken genomics. The B and WL hens were reared in cages on 14-h light/d, and at 2 wk after they had begun to lay, at 24 to 26 wk, were transferred in groups of six to seven, to pens (4 × 1 m) each of which contained eight nest boxes on the floor. Each nest box contained wood shavings as nesting material and four to five hard-boiled eggs to encourage incubation behavior. When the hens were transferred to floor pens the light intensity was reduced from 25 to 12 lx, and the photoperiod was increased to 16 h light/d. Fresh laid eggs were removed daily; broken or lost hard-boiled eggs were replaced with hard-boiled eggs. The ambient temperature was maintained at 18 to 23 C. The birds had free access to feed and water at all times.

Twice-daily records were kept of nesting behavior. Hens that were observed to be persistently nesting for 3 to 4 consecutive d and that were clucking and raising their feathers when approached were recorded as incubating. Observations on incubation behavior were made for 28 wk after housing in floor pens. At the end of this period any hens that had not become broody were transferred to 8 h light/d and individually caged with feed and water freely available. This procedure resulted in the complete cessation of egg laying. After a further 10 wk, the photoperiod was increased to 16 h light/d, and the hens were transferred back to floor pens as previously described, after they had resumed laying, to record the incidence of incubation behavior for a further 28 wk.

Observations were made on the incidence of incubation behavior in hens from five different types of mating:

- Group 1 (n = 28). Daughters of three B hens naturally mated to three B cockerels.
- Group 2 (n = 28). Daughters of three WL hens naturally mated to three WL cockerels.
- Group 3 (n = 73). Daughters of two B hens artificially inseminated with semen from a WL cockerel. The two B hens were initially selected because they showed incubation behavior and were recycled into a second egg laying period by transfer to 8 h light/d and back to 16 h light/d.
- Group 4 (n = 37). Daughters of two WL hens artificially inseminated with semen from a B cockerel.
- Group 5 (n = 103). Daughters of four WL hens artificially inseminated with semen from one of the F_1 males from the parents used to generate Group 3 (male WL × female B).

Statistical Analyses

As a first approximation, the hypothesis tested was that incubation behavior was controlled by a single dominant gene on the Z chromosome. A chi-squared test (Mead and Curnow, 1983) was applied to assess whether or not a difference between a predicted and observed incidence of broodiness was significant. The actual incidence of the trait measured in the B population was 78.6%, which was taken into account when analyzing the phenotype segregation in the progeny from three experimental matings. The data were also analyzed in the same way to test the alternative hypothesis that incubation behavior was controlled by a single dominant autosomal gene.

RESULTS

The incidence of incubation behavior in the offspring from the five groups of different matings is shown in Table 1. The incidence of incubation behavior in Group 1, the B × B mating, was 78.6% whereas in Group 2, the WL × WL mating, there was no incubation behavior. Assuming the presence of single dominant broody gene on the Z chromosome (major sex linkage), the incidence of incubation behavior in the male WL × female B cross should be 0%, which was less than the observed incidence of 61.6%. Conversely, making the same assumption, the

TABLE 1. Observed and predicted incidence of incubation behavior in White Leghorns (WL), Bantams (B), and their crosses assuming major sex-linked or permissive autosomal genes control of incubation behavior and that their expression results in 78.6% incubation behavior in the environmental conditions to which all groups were exposed

Prediction tested	Group no.	Mating	Hens incubating/total (n)	Percentage incubating	
				Predicted	Observed
None	1	♂ B × ♀ B	22/28	. . .	78.6
None	2	♂ WL × ♀ WL	0/28	. . .	0
Major sex-linkage	3	♂ WL × ♀ B	45/73	0	61.6***
	4	♂ B × ♀ WL	21/37	78.6	56.8*
	5	♂ F_1 (♂ WL × ♀ B) × ♀ WL	6/103	39.3	5.8***
Major permissive autosomal	3	♂ WL × ♀ B	45/73	78.6	61.6***
	4	♂ B × ♀ WL	21/37	78.6	56.8*
	5	♂ F_1 (♂ WL × ♀ B) × ♀ WL	6/103	39.3	5.8***

*$P > 0.05$, ***$P > 0.001$ for the difference between predicted and observed.

incidence of incubation behavior in the male B × female WL cross should be much greater than in the male WL × female B cross and approach that in the B hens. These predictions were incorrect as there was no significant difference between the incidences of incubation behavior between the WL by B reciprocal crosses. Again, assuming the presence of a single dominant broody gene on the Z chromosome, in the F_1 backcross (Group 5) the incidence of broodiness should have been 39.3% (i.e., half of the expressed broodiness incidence in the Group 1), which was significantly greater than the observed 5.8%.

In the reciprocal crosses (Groups 3 and 4), 61.6% hens with a WL father and 56.8% of hens with a B father showed incubation behavior ($P > 0.05$, NS). The corresponding times to the onset of incubation behavior after transfer to floor pens were 86.19 ± 5.33 and 78.00 ± 8.17 d, respectively ($P > 0.05$, NS, Student's *t*-test).

Assuming the presence of major permissive autosomal broody genes and no sex-linked broody genes, the incidence of incubation behavior in Groups 3 and 4 was predicted to be the same as in Group 1, and in Group 5 it was predicted to be about the same as that in Group 1. None of these predictions were realized. Observations were made on morphological features to establish whether there was any correlation with the expression of incubation behavior in the F_1 backcross. The phenotypes of the six F_1 backcross hens showing incubation behavior were as follows: white skinned, 4; black skinned, 2; single comb, 3; duplex comb, 3; white feathered, 2; white feathered speckled black, 2; white and black feathered, 2; smooth feathered, 3; coarse feathered, 3; and four toes, 6. No combination of these phenotypes appeared to be associated with the expression of incubation behavior.

DISCUSSION

These data are inconsistent with the hypothesis that incubation behavior is a multigene trait with a major sex-linked component. Saeki (1957) and Saeki and Inoue (1979) found that broodiness in crosses of WL males with broody stock females was 11.1 to 45.4% and that in the reciprocal cross it was 63.0 to 85.2%. In similar reciprocal crosses, in the current study, the corresponding incidences of incubation behavior of 61.6 and 56.8% were observed. A possible explanation for this discrepancy may be that the design of the experiments described by Saeki (1957) and Saeki and Inoue (1979) did not take fully into account the phenomenon of deferred broodiness, which might be characteristic of the daughters of WL males (Goodale et al., 1920; Hays, 1940). The finding in the present study does not support this view that the daughters of WL males did not require more time to show incubation behavior than the daughters of B males. Further evidence against the expression of incubation behavior being controlled by sex-linked genes comes from observation on the incidence of incubation behavior in the F_1 backcross. Here, the presence of sex-linked genes would have been predicted to have resulted in an incidence of incubation behavior of about half (39.3%) that of the B grandparents. This prediction was not supported as the incidence of incubation behavior in the F_1 backcross was only 5.8%. In view of these findings, an alternative hypothesis that incubation behavior is controlled by a dominant autosomal gene (or genes) should be considered. A re-analysis of the data assuming this hypotheses showed it to be unlikely again due to a very low incidence of broodiness in the F_1 backcross.

The limited amount of data available for the F_1 backcross provided no evidence that the expression of incubation behavior might be linked to several morphological characteristics. If any such correlation had been found, a broody trait gene might be expected to map close to a gene controlling the morphological character.

Because the present data were not consistent with the view that the expression of incubation behavior is controlled by dominant permissive autosomal genes or by Z linked genes, an alternative explanation should be considered. As a first approximation, the current data suggest that broodiness was controlled by a dominant autosomal gene at one locus in the B and a nonbroody autosomal gene at another locus in the WL. Assuming *A* be an incompletely dominant gene for broodiness and *B* an incompletely dominant inhibitor of broodiness, the parents and F_1 progeny in the test and reciprocal crosses would have the following genotypes:

♂ WL × ♀ B
aaBB *AAbb*
↓
F_1 ♂ *AaBb*; ♀ *AaBb*

♂B × ♀ WL
AAbb *aaBB*
↓
F_1 ♂ *AaBb*; ♀ *AaBb*

Assuming an incomplete dominance of both genes and variable environmental effects on the broody trait, the incidence of incubation behavior in the test and reciprocal crosses is predicted to be less than in the B grandparents (78.6%), which is consistent with the observed values (61.6 and 56.8%, respectively).

In the backcross progeny, the genotype segregation would be as follows:

♂F_1 (♂ WL × ♀ B) × ♀ WL
AaBb *aaBB*
↓
♂ ¼ *AaBB*, ¼ *AaBb*, ¼ *aaBB*, ¼ *aaBb*;
♀ ¼ *AaBB*, ¼ *AaBb*, ¼ *aaBB*, ¼ *aaBb*

In this backcross progeny, incubation behavior would be expected in female diheterozygotes (*AaBb*) resulting in an incidence of broodiness of about 25%. Assuming an incomplete dominance of both genes, partial expression of broodiness in the B hens and variable environmental effects on the broody trait, the incidence of incubation behavior in the test and backcrosses should be less than 25%. The observed percentage of broodiness was 5.8%.

If more incompletely dominant genes and inhibitors, possibly including sex-linked ones, and some other additive genes with smaller effects (both positive and negative) are involved in this complex interaction and there is a variable environmental influence on the expression of incubation behavior, the theoretical percentages might fit empirical figures.

In conclusion, the present observations are consistent with the view that incubation behavior in chickens is not controlled by a major gene (or genes) on the Z chromosome. There must, therefore, be major autosomal genes contributing to the expression of the behavior. If a broody gene exists on the Z chromosome, it might be one of at least three genes including two incompletely dominant autosomal genes, one causing and other one inhibiting incubation behavior, probably with equal influence.

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REFERENCES

Buntin, J. D. 1996. Neural and hormonal control of parental behavior in birds. Adv. Stud. Behav. 25:161–213.

Dunn, I. C., G. McEwan, T. Okhubo, P. J. Sharp, I. R. Paton, and D. W. Burt. 1998. Genetic mapping of the chicken prolactin receptor gene: A candidate gene for the control of broodiness. Br. Poult. Sci. 39:S23–24.

Goodale, H. D., R. Sanborn, and D. White. 1920. Broodiness in domestic fowl. Massachusetts Agric. Exp. Sta. Bull. 199. Agricultural Experimental Station, Amherst, MA.

Hays, F. A. 1940. Inheritance of broodiness in Rhode Island Reds. Massachusetts Agric. Exp. Sta. Bull. 377. Agricultural Experimental Station, Amherst, MA.

Hays, F. A., and R. Sanborn. 1939. Breeding for egg production. Massachusetts Agric. Exp. Sta. Bull. 307. Agricultural Experimental Station, Amherst, MA.

Hutt, F. B. 1949. Genetics of the Fowl. McGraw-Hill Book Company, Inc., New York.

Mead, R., and R. N. Curnow. 1983. Statistical Methods in Agriculture and Experimental Biology. Chapman and Hall, London.

Ohkubo, T., M. Tanaka, K. Nakashima, R. T. Talbot, and P. J. Sharp. 1998. Prolactin receptor gene expression in the brain and peripheral tissues in broody and non-broody breeds of domestic hen. Gen. Comp. Endocrinol. 109:60–68.

Romanov, M. N., R. T. Talbot, P. W. Wilson, and P. J. Sharp. 1999. Inheritance of broodiness in the domestic fowl. Br. Poult. Sci. 40:S20–S21.

Saeki, Y. 1957. Inheritance of broodiness in Japanese Nagoya fowl, with special reference to sex-linkage and notice in breeding practice. Poult. Sci. 36:378–383.

Saeki, Y., and Y. Inoue. 1979. Body growth, egg production, broodiness, age at first age and egg size in Red Jungle fowls, and attempt at their genetic analyses by the reciprocal crossing with White Leghorns. Jpn. Poult. Sci. 16:121–125.

Sharp, P. J., M. C. Macnamee, R. J. Sterling, R. W. Lea, and H. C. Pedersen. 1988. Relationships between prolactin, LH and broody behavior in Bantam hens. J. Endocrinol. 18:279–286.

Tanaka, M., K. Maeda, T. Ohkubo, and K. Nakashima. 1992. Double antenna structure of chicken prolactin receptor deduced from the cDNA sequence. Biochem. Biophys. Res. Commun. 1888:490–496.